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THE TRANSPORT OF CO₂ IN THE BLOOD OF CERTAIN FRESHWATER FISHES

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The effect of carbon dioxide (and other acids) on the affinity of the hemoglobin in fish blood for oxygen varies greatly from species to species (Redfield, 1933). In some species not only the affinity of the blood for oxygen, but the oxygen capacity too, is greatly diminished by relatively low pressures of CO₂ (Root, 1931). The physiological and ecological implications of great differences in affinity for oxygen and sensitivity to CO₂ have become more apparent as a result of a series of studies on freshwater fish, which are summarized in a recent paper by Black (1940). A few of the findings may be recapitulated briefly as follows. Carbon dioxide decreases the affinity of the blood for oxygen to a greater degree in those fish inhabiting deeper and colder water. The same bloods have also a lower affinity for oxygen at minimal pressures of CO₂. These two characteristics would act to offset the effect of low temperatures, which is to lower the pressure at which oxygen is available to the tissues. Another manner in which sensitivity to CO₂ may be useful to fish inhabiting deep water, is in the regulation of buoyancy at different depths, by facilitating the formation in the swim-bladder of gases rich in oxygen, as suggested by Haldane (1922) and Hall (1924).

The present study was designed with two objects in mind. The first was to investigate the mechanisms by which the great differences in sensitivity to CO₂ are achieved. The second was to determine the probable range of physiological tensions of CO₂ in a series of freshwater fish comprising some of those used in the foregoing studies. This information is difficult to obtain with any degree of accuracy in fish, but even approximate determinations may be of use in evaluating some of the relationships described above.

In pursuit of the first object, a detailed study of the characteristics



of CO₂ transport was made on the blood of two species differing widely in the sensitivity of their blood to CO₂, namely rainbow trout, *Salmo gairdnerii* Richardson, and carp, *Cyprinus carpio* Linnaeus. Trout are among the fish most sensitive to CO₂ and carp, while not the most insensitive, are relatively so. Availability in sufficient numbers to supply enough blood was an important consideration in the choice of these species. Data on physiological CO₂ tensions were obtained on the same species as well as on a few specimens of bullhead, *Ameiurus nebulosus* Le Sueur, and sucker, *Catostomus commersonnii* Lacepède, which happened to be available. The blood of the bullhead is even less sensitive to CO₂ than that of the carp, while those of the sucker and the trout are of about the same order of sensitivity.

METHODS

Blood was drawn from the heart into a syringe containing heparin. No fluoride or oxalate was used, since these have been found to cause progressive swelling of the erythrocytes and eventual hemolysis in certain fish bloods (Black and Irving, 1938; Hamdi and Ferguson, 1940). For the construction of dissociation curves it was necessary to pool the blood from several fish. Equilibration was done at 15° C., except in specified cases. The blood was kept on ice throughout the day. Samples were equilibrated one at a time for fifteen minutes in tonometers of the original Barcroft type (1914). A centrifuge tube was attached to the open end of the tonometer by rubber tubing. After equilibration the blood was collected in the centrifuge tube and separated from the gas phase by clamping the rubber tubing. The gas was analyzed for CO₂ and O₂ in a Haldane apparatus. The blood in the centrifuge tube was covered with liquid paraffin and samples were removed without delay for analysis of CO₂ and O₂ by the manometric method of Van Slyke; of chloride by the open Carius method; and of water content by drying at 105° C. Another sample was centrifuged in a capillary tube for twenty minutes at a centrifugal force of about 3000 times gravity for the estimation of packed cell volume. The rest of the blood was also centrifuged under oil for 20 minutes and samples of plasma removed for the analyses listed above. A number of experiments were performed to evaluate the error introduced by metabolism of the blood during the foregoing procedures. Hourly analyses were done on blood kept in sealed syringes at 15° C. and on ice. Trout blood was found to have a somewhat higher metabolic rate than carp blood, and the highest rate found in trout blood at 15° C. was 1 cc. of oxygen consumed and 1 cc. of CO₂ produced per 100 cc. blood per hour. The only estimation which

might have been appreciably affected by even this highest metabolic rate was the estimation of cell volume in which centrifuging was done at 25° C. or thereabouts for 20 minutes. It was possible in the later experiments on trout blood to minimize this source of error by reducing the time of centrifuging to 5 minutes in a high speed hematocrit centrifuge. The results with both long and short times of centrifuging were in substantial agreement.

In trout blood kept on ice, the highest metabolic rate was found to be 0.5 cc. per 100 cc. per hour. This figure may be used to estimate the maximum correction for the figures on O₂ and CO₂ content of the venous blood of trout, because the trout blood was kept on ice for three or four hours in transit from the hatchery to the laboratory.

Some experiments were done to determine the change in CO₂ capacity with time. No changes were found in blood kept on ice for as long as 12 hours. When the blood was kept at 10° C. in a refrigerator for 24 to 48 hours, small changes occurred. It is interesting that the CO₂ capacity increased in blood (both carp and trout) kept fully oxygenated, but decreased in blood which was partly or fully reduced, suggesting something like a Pasteur reaction.

The gas content of mixed venous blood, drawn from the heart into an oiled syringe containing heparin, was determined by analysis without exposure to air. Three portions of the same sample were then equilibrated with different gas mixtures and subsequently analyzed. One portion was equilibrated with about 2 mm. CO₂ in air. This gave the oxygen capacity and one point on the CO₂ dissociation curve. Another portion was equilibrated with 8–12 mm. CO₂ in air and the third portion with 8–12 mm. CO₂ in N₂. These gave the positions of the CO₂ dissociation curves of oxygenated and reduced blood respectively. The equilibrations in these particular experiments were done at the temperature of the water from which the fish were taken. Knowing the oxygen content of the sample of venous blood and the oxygen capacity of the same sample, it was possible to estimate the position of the CO₂ dissociation curve of the venous blood between those of the fully oxygenated and fully reduced bloods. Then knowing the CO₂ content of the venous blood, its CO₂ tension could be read off on the abscissa with an error probably not more than 2 or 3 mm. Hg.

During the withdrawal of the venous blood, the gills were not aerated, but only samples which flowed freely into the syringe at the first stab were used. Since even the trout survived this procedure without apparent harm, the estimated tensions may be regarded as well within the limit of tolerance of the fish, though probably above the average for the resting state.

OBSERVATIONS

Physiological Gas Tensions

The results on the gas contents and tensions in circulating blood are presented first, since they indicate the part of the CO₂ dissociation curves of greatest physiological importance. Table I gives the CO₂ content, percentage saturation with oxygen and estimated CO₂ tensions of samples of venous blood drawn from four species of fish. Only the trout blood could have changed significantly between the time of drawing the blood and the time of analysis. If maximum corrections for metabolism are applied to the results on trout blood, the CO₂ tension would be about

TABLE I
Venous blood gases

Trout	Temperature	CO ₂	O ₂	P CO ₂
	°C.	v.p.c.	per cent sat.	mm. Hg
<i>B</i>	22	21.0	0	9
<i>C</i>	22	24.8	3	10
<i>D</i>	15	19.4	0	8
<i>E</i>	15	22.8	0	10
Carp				
<i>J</i>	10	36.4	47	5
<i>G</i>	15	31.2	30	10
<i>H</i>	15	28.6	18	7
Sucker				
<i>A</i>	8	47.8	37	9
<i>B</i>	8	36.3	26	7
Catfish				
<i>A</i>	8	21.4	62	8

1 mm. lower, the CO₂ contents about 2 vols. p.c. lower and the oxygen contents about 2 vols. p.c. higher.

No measurements were made on the aerated blood from the gills, but a rough estimate of the CO₂ tension in aerated blood can be made in the following manner. Trout *D* in Table I is favorable for purposes of illustration, because it had practically the same O₂ and CO₂ capacity as the composite blood *F* in Fig. 1. If the blood of trout *D* were fully oxygenated, it would gain about 11 vols. p.c. of O. Assuming an R.Q. of unity, the CO₂ lost would be 11 vols. p.c., leaving a CO₂ content of about 8 vols. p.c. A CO₂ content of 8 vols. p.c. corresponds on the curve for oxygenated trout blood in Fig. 1 to a CO₂ tension of about 3.5 mm. Similar calculations for all the bloods in Table I gave estimated

CO₂ tensions of 3–5 mm. for fully oxygenated "arterial" blood. These figures indicate that the most important range of physiological CO₂ pressure is between 3 and 10 mm. Hg in all four species. It is a matter of considerable interest that the physiological range is so similar in fish representing the extremes of variation in the effect of CO₂ on the combination of oxygen in the blood. It may be concluded that the consequences of differences in sensitivity to CO₂ are not evaded to any extent, as might conceivably be the case if different species maintained themselves at significantly different CO₂ pressures.

Another implication of the foregoing results is that CO₂ tensions above 10 mm. could only be attained in any of these fish under condi-

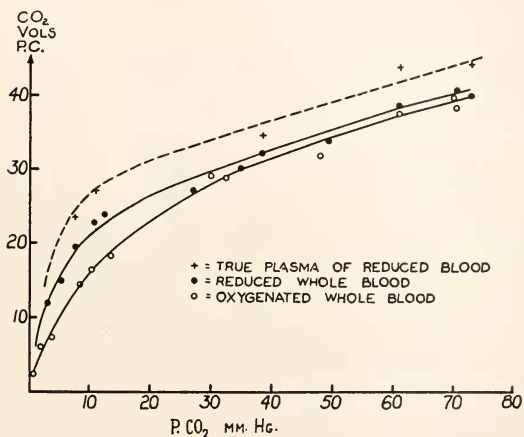


FIG. 1. CO₂ dissociation curves of pooled blood of rainbow trout at 15° C.

tions of oxygen debt. Higher tensions might occur, however, in certain tissues as a result of acid production locally. Consequently the rather low CO₂ tensions found in mixed venous blood need not be regarded as the maximal CO₂ tensions which may operate in the production of gases in the swim-bladder in deep water.

CO₂ Dissociation Curves

Figure 1 shows composite curves of two batches of blood each from twelve rainbow trout. They resemble the curves obtained by Root (1931) and Root and Irving (1940) on various marine fish in the con-

vergence of the curves for oxygenated and reduced blood at the higher pressures of CO_2 . It can be seen in Table II that the oxygenated blood is not fully oxygenated at the higher pressures of CO_2 even though in some cases it was exposed to pressures of oxygen as high as 670 mm. Hg.

The apparent disappearance of the effect of oxygenation on CO_2 capacity (Haldane effect) at the higher pressures of CO_2 can be due only in part to incomplete oxygenation of the oxygenated blood because even at the high pressures it is still half saturated with O_2 .

In Fig. 2 are shown the CO_2 dissociation curves of carp blood. Two sets of curves are given representing the range of variation found in six batches of carp blood. The greater variation shown by the carp may be due in part to the use of fewer fish for each batch of blood (the carp

TABLE II

Complete data on blood of trout F at 15°C. To calculate combined O_2 (Hb O_2) the Bunsen solubility coefficient of oxygen in the blood at 15° is assumed to be 0.036. The meaning of $r \text{ CO}_2$ and $r \text{ Cl}$ is explained in the text.

P CO_2	PO_2	CO_2 content		Hb O_2	$r \text{ CO}_2$	Cell vol.	Chloride		$r \text{ Cl}$	Water content	
		Whole blood	Plasma				Whole blood	Plasma		Whole blood	Plasma
mm. Hg	mm. Hg	v.p.v.	v.p.c.	v.p.c.		per cent	m. eq./l.	m. eq./l.		g./100 g.	g./100 g.
2.2	Air	6.1	8.0	9.9	0.33	32.8	—	140.0	0.60	86.0	94.5
2.7	0	11.9	13.8	0.0	0.71	33.0	116	139.5	0.61	86.0	94.6
10.7	124	16.3	18.0	7.3	0.90	35.3	118	140.0	0.65	86.5	95.5
12.5	0	23.8	26.4	0.0	0.95	47.0	117	136.0	0.81	85.9	94.3
32.7	168	28.6	31.5	5.0	0.94	43.0	116	138.0	0.78	86.0	94.9
38.5	0	31.8	34.2	0.0	1.02	44.0	117	139.5	0.77	85.8	94.5
61.0	675	37.3	40.6	5.5	0.94	37.4	116	139.0	0.68	85.3	94.6
70.5	0	40.4	44.0	0.0	0.98	43.8	116	138.0	0.77	84.8	95.0

being larger fish), and consequently less averaging out of individual variations. The curves for carp differ from those of the trout in two important respects. Firstly they are higher, indicating a higher pH at a given pressure of CO_2 . Secondly the curves of oxygenated and reduced bloods are widely separated and show only a slight tendency to converge at higher CO_2 pressures.

The higher CO_2 capacity of carp blood indicates a higher pH which must be attributed to the regulation of the acid-base balance of the fish as a whole at a more alkaline level. The CO_2 dissociation curves of true plasma in both species lie above the curves for whole blood, in this respect resembling mammalian blood rather than dogfish blood (Ferguson, Horvath and Pappenheimer, 1938). They indicate a higher concentration of CO_2 in the plasma than in cells at the same pressure of

CO₂. The distribution of CO₂ between cells and plasma is more precisely indicated in Table II in the column headed r CO₂.

Distribution of Electrolytes

The distribution ratio r CO₂ is the concentration of combined CO₂ per gram of cell water divided by the combined CO₂ per gram of plasma water. Combined CO₂ is calculated by subtraction of the physically dissolved CO₂ from the total CO₂, using the factors 0.125 and 0.105 for plasma and cells respectively, which multiplied by the PCO₂ in mm. Hg give the concentrations of dissolved CO₂ in volumes per cent. RCI is

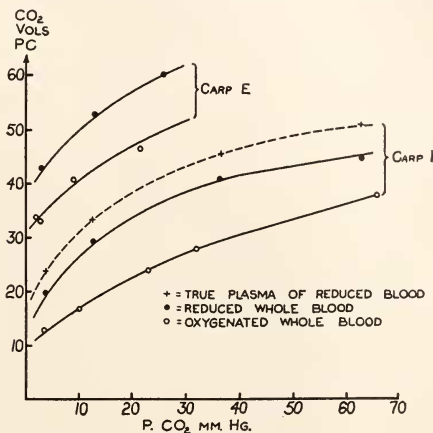


FIG. 2. CO₂ dissociation curves of carp blood at 15° C. Of six batches of pooled carp blood, E had the highest CO₂ capacity and I the lowest.

the analogous distribution ratio for chlorides. Little weight should be given to individual values of the distribution ratios because of the large number of possible errors that are introduced in their computation. On the average, however, it is evident that in carp blood the values of r CO₂ do not tend to exceed those of r Cl as they do in mammalian blood where the higher value of r CO₂ is probably due mostly to the presence of carbamino compounds of CO₂ with hemoglobin (Roughton, 1935). In the trout blood the values of r CO₂ do tend to exceed somewhat the values of r Cl. Thus the distribution ratios provide no evidence for the presence of carbamino compounds in the carp erythrocytes, but would be consistent with the presence of a small amount in trout cells. They

are consistent with the hypothesis that the combined CO_2 in red cells is largely in the form of bicarbonate and that the bicarbonate and the chloride are partitioned between the red cells and plasma according to a Donnan distribution. The figures on plasma chloride of carp blood in Table III give further evidence of a Donnan equilibrium with chloride and bicarbonate ions diffusible. The plasma chloride decreases regularly (except for one figure) with each increase in plasma CO_2 consistent with a migration of chloride into the cells with increasing acidity. Plasma chloride in trout blood also shows a decrease with increasing CO_2 , but in a very irregular fashion which, however, acquires more meaning when considered with the changes in packed cell volume.

TABLE III
Complete data on blood of carp I at 15°C.

P CO_2	PO_2	CO_2 content		Hb O_2	r CO_2	Cell vol.	Chloride		r Cl	Water content	
		Whole blood	Plasma				Whole blood	Plasma		Whole blood	Plasma
<i>mm. Hg</i>	<i>mm. Hg</i>	<i>v.p.c.</i>	<i>v.p.c.</i>	<i>v.p.c.</i>		<i>per cent</i>	<i>m. eq./l.</i>	<i>m. eq./l.</i>		<i>g./100 g.</i>	<i>g./100 g.</i>
3.4	158	12.9	15.2	12.3	0.77	39.6	124.5	146.0	0.94	82.5	94.8
3.7	2.3	19.8	23.8	1.8	0.77	41.2	125.0	144.0	0.88	83.9	94.8
10.5	148	17.0	21.2	12.7	0.61	38.8	125.0	146.5	0.90	84.1	95.1
12.6	0	29.3	33.7	0.4	0.88	40.6	125.5	142.5	0.91	83.5	94.6
32.2	145	27.9	31.6	10.8	0.90	39.5	125.5	144.5	0.85	83.2	95.0
36.6	0	41.1	45.9	0	0.95	40.2	126.0	141.5	0.94	83.4	94.5
66.2	224	38.1	42.2	10.8	0.98	40.9	125.0	142.0	0.92	83.6	94.4
63.2	0	45.0	51.3	0	0.88	41.2	124.5	140.5	0.95	82.7	95.2

Cell Volumes

In Fig. 3 the packed cell volumes of carp and trout blood are plotted against pressure of CO_2 . In carp blood the cell volume decreases slightly with the first increment of CO_2 pressure, but with further increase in P CO_2 the volume increases as in mammalian blood, and by about the same amount for each increment of CO_2 combined, namely by about 5 p.c. of their volume for an increase of 10 m.eq. per liter of combined CO_2 . The cells of T.J.F. (Henderson, 1928) increased by 6 per cent for an increase of 10 m.eq. of CO_2 combined.

The volume changes for trout blood shown in Fig. 3 are the composite data from three batches of blood. The trout cells show greater changes in volume than do the carp cells. They reach their maximum size between 10–20 mm. Hg of CO_2 pressure. The reduced cells show a much greater increase in volume, but at pressures above 20 mm. Hg they decrease again with increasing P CO_2 . It can be seen in Table II

that the largest cell volume corresponds with the lowest concentration of chloride in the plasma, suggesting that a migration of chloride from the plasma, presumably into the cells, has occurred. The extent of this migration is depicted in Fig. 4, where the chloride content in the plasma of a liter of blood is plotted against the bicarbonate content of the plasma of a liter of blood. The contents are calculated by multiplying the concentrations per liter of plasma by the fraction of the whole blood volume which is plasma. In carp blood it appears that for each increase of 1 eq. of bicarbonate in the plasma about 0.75 eq. of chloride enters the cells. This ratio is approximately that for mammalian blood (Van

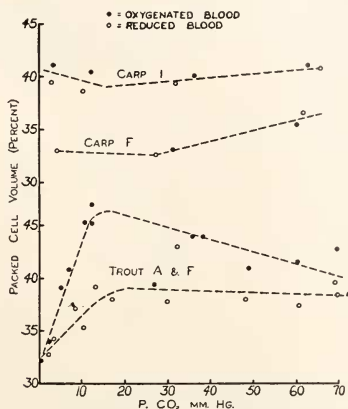


FIG. 3. Packed cell volumes in carp and trout blood are plotted against the pressure of CO₂. The large changes in cell volume in trout blood are in striking contrast to the small changes in carp blood.

Slyke, 1921). In trout blood, however, the loss of chloride from the plasma at the maximum cell volume greatly exceeds the increase in CO₂ in the plasma. This suggests that the excessive cell volumes and excessive chloride shift may be due to the production in the blood of an acid other than CO₂. It is immaterial where this acid is produced, but it is necessary to postulate that it is diffusible through the red cell membrane. It is also necessary to postulate that it is produced by a reversible reaction and that the equilibrium point is determined by the tension of oxygen and of CO₂ in the blood. The optimum conditions for its formation would presumably be at low oxygen tensions and a CO₂ tension between 10 and 20 mm. To explain the cell volume changes by



this production of acid alone would require the formation of about 30 m.eq. per liter of acid other than CO_2 . Such an hypothesis certainly deserves the utmost skepticism till the changes in cell volume can be checked by a method other than centrifuging, but it does receive some support of a qualitative nature from another and independent consideration, namely the effect of oxygenation on CO_2 capacity.

Oxygenation and CO_2 Capacity

When the effect of oxygenation on CO_2 capacity is expressed as $-\Delta\text{BHCO}_3/\Delta\text{O}_2$ at constant plasma pH the maximum values for this

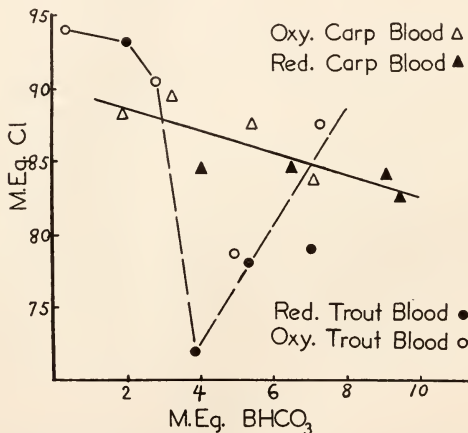


FIG. 4. The chloride in the plasma of a liter of blood is plotted against the bicarbonate in the same volume to illustrate the magnitude of the migration of chloride into the cells. In carp blood $\Delta\text{BHCO}_3/\Delta\text{Cl} = -.75$ as in mammalian blood, but in trout blood the ratio is much greater than unity, suggesting that an acid other than CO_2 has been neutralized in the cells and diffused into the plasma in exchange for chloride ions.

ratio are found at a pH of about 7.3 in both bloods. The maximum value in each is about 1.2. This value is higher than any of those in other species compiled by Redfield (1933), as it should be, in accordance with the principle that the greater the effect of acid on the combination of oxygen the greater should be the effect of oxygenation on the dissociation of hemoglobin as an acid. But the fact that the value is the same in trout and carp blood is apparently inconsistent with the principle. If, however, the hypothesis of "extra acid" production in trout

blood is correct, the apparent inconsistency would be only apparent, for if an acid other than CO₂ were produced in the blood at low pressures of oxygen, ΔBHCO_3 would not be a complete measure of the change in base combined with hemoglobin, and hence would give too low an estimate of the effect of oxygenation on the acid dissociation of the hemoglobin in trout blood.

Buffer Power

The buffer power (β) of whole blood is often represented by the ratio $-\Delta\text{BHCO}_3/\Delta\text{pH}_s$, where ΔBHCO_3 is the change in CO₂ combined in whole blood and pH_s the pH of the plasma or serum. This procedure will be erroneous in trout blood if the hypothesis of the extra acid production is correct. It is interesting, however, to make the cal-

TABLE IV
Buffer power of reduced bloods.

	Range of plasma pH	Range of P CO ₂	Buffer power (β)
Carp 15°	8.11-7.36	2-20	1.6
	7.36-6.96	20-60	1.4
Trout 15°	7.90-7.23	2-20	2.3
	7.23-6.85	20-60	1.7
*Human 38°	7.5-7.2	35-90	2.3

* Peters and Van Slyke, *Quantitative Clinical Chemistry*, Vol. I.

$\beta = -\Delta\text{BHCO}_3/\Delta\text{pH}/\text{Hb}$. Concentrations are in milliequivalents per liter.

culatation in the conventional manner and then to consider what change in conclusion would be necessary if the hypothesis of extra acid production were correct. The calculated buffer powers of carp, trout and human blood adjusted to equal concentrations of hemoglobin are given in Table IV. Figures are given for reduced blood only, since fully oxygenated trout blood cannot be obtained over a large enough range of pH. Carp blood has about the same buffer power over the range studied, but trout blood shows a greater buffer power over the more physiological range of P CO₂ of 2-20 mm., where it is equal to the buffer power of human hemoglobin. Over the higher range of CO₂ pressure the buffer power of trout hemoglobin is less, though still higher than that of carp blood. The trout blood shows a concentration of its buffer power in the physiological range, a characteristic which may be attributed tentatively to closer grouping of the dissociation constants of

the acid (or basic) groups in this range. If the anomalous increase and decrease in cell volume in trout blood represents increase and decrease in extra acid, the true buffer power over the range 2–20 mm. would be even greater, and even less over the range 20–60 mm. In other words, the tendency for the buffer power to be concentrated in a narrow range in the trout blood would be even more striking.

Plasma pH

Values of plasma pH at CO_2 pressures of 2, 20 and 60 mm. Hg calculated from the smoothed data of the dissociation curves, assuming a pK_1' of 6.2 are shown in Table V. The changes in plasma pH may be taken as paralleling fairly closely the changes in cellular pH. To calculate these separately would merely introduce the uncertainties of arbitrary values for pK_1' in cells.

The main points of interest are: (1) an increase of P CO_2 from 2 mm. to 20 mm. produces a greater change in pH in carp blood than

TABLE V

pH of true plasma of oxygenated blood calculated from the data of Tables II and III.

P CO_2 <i>mm. Hg</i>	Carp	Trout
2	7.91	7.66
20	7.23	7.15
60	6.84	6.84

in trout blood; (2) at P CO_2 of 60 mm. the carp blood is as acid as the trout blood. In other words, the loss in oxygen capacity in trout blood cannot be attributed either to a greater change in acidity for a given increase in P CO_2 or even to a higher absolute acidity at the higher pressures of CO_2 . However, the lower acidity in carp blood at lower pressures of CO_2 must be held partly responsible for the higher affinity of the blood for oxygen in the absence^o of CO_2 .

DISCUSSION

The results as a whole indicate that the great differences in the effect of CO_2 on the combination of oxygen in these two bloods and their affinity for oxygen may be due to adaptations at three levels of physiological organization. These seem to be: (1) specific differences in the hemoglobin molecule; (2) differences in the environment provided for the hemoglobin by the erythrocyte; (3) differences in the acid-base regulation of the fish as a whole. It seems likely that differences in the

hemoglobin molecules will prove to be the most important element in the total adaptation, although no comparison of the two hemoglobins in solution has yet been made. Until such a comparison is made, it cannot be said that differences in the erythrocytes may not be equally important. Certainly one of the most striking differences between the bloods has been the behavior of the cell volumes. The effect of the intracellular environment on the affinity for oxygen is marked, even among mammals, and varies from species to species (Hill and Wolwekamp, 1936) in a manner as yet inexplicable. In fish blood the effect of hemolysis on affinity for oxygen is also large and cannot be explained by changes in acidity alone (Root and Irving, 1940). The anomalous behavior in trout blood of cell volumes, plasma chlorides and effect of oxygenation on CO₂ capacity could all be explained, at least in part, by the production (by a kind of Pasteur reaction) of acid at low tensions of oxygen and an optimal tension of CO₂. The production of extra acid cannot, however, explain the loss of oxygen capacity or the convergence of the CO₂ dissociation curves of oxygenated and reduced blood. At most it could only be a mechanism augmenting the effect of CO₂ in maintaining a high tension of oxygen at low contents of oxygen in the blood of trout.

SUMMARY

The venous blood from four species of freshwater fish, rainbow trout (*Salmo gairdnerii* Richardson), carp (*Cyprinus carpio* Linnaeus), bull-head (*Ameiurus nebulosus* Le Sueur) and sucker (*Catostomus commersonnii* Lacepède) was analyzed and an estimate made of the probable range of physiological CO₂ tension.

A detailed study was made of CO₂ transport in the blood of two of these species, the rainbow trout and the carp, which differ greatly in the effect of CO₂ on the combination of oxygen in the blood. They differ too, in their systems of CO₂ transport. A curious feature of trout blood is the great change in packed cell volume with changes in the pressure of O₂ and CO₂. An hypothesis is presented to explain in part these anomalous changes in cell volume and other characteristics of the trout blood. Carp blood shows less differentiation from general vertebrate characteristics.

LITERATURE CITED

- BARCROFT, J., 1914. The Respiratory Function of the Blood. Cambridge.
BLACK, E. C., 1940. The transport of oxygen by the blood of freshwater fish. *Biol. Bull.*, 79: 215-229.
BLACK, E. C., AND LAURENCE IRVING, 1938. The effect of hemolysis upon the affinity of fish blood for oxygen. *Jour. Cell. and Comp. Physiol.*, 12: 255-262.

- BLACK, E. C., F. E. J. FRY AND W. J. SCOTT, 1939. Maximum rates of oxygen transport for certain freshwater fish (abstract). *Anat. Rec.*, **75**: (Supplement) 80.
- FERGUSON, J. K. W., S. M. HORVATH AND J. R. PAPPENHEIMER, 1938. The transport of carbon dioxide by erythrocytes and plasma in dogfish blood. *Biol. Bull.*, **75**: 381-388.
- HALDANE, J. S., 1922. Respiration. New Haven.
- HALL, F. G., 1924. The functions of the swimbladder of fishes. *Biol. Bull.*, **47**: 79-126.
- HAMDI, T. M., AND J. K. W. FERGUSON, 1940. Hemolytic action of fluorides on certain nucleated erythrocytes. *Proc. Soc. Exp. Biol. and Med.*, **44**: 427-428.
- HENDERSON, L. J., 1928. Blood; a Study in General Physiology. New Haven.
- HILL, R., AND H. P. WOLVEKAMP, 1936. The oxygen dissociation curve of haemoglobin in dilute solution. *Proc. Roy. Soc. London, B*, **120**: 484-495.
- PETERS, JOHN P., AND DONALD D. VAN SLYKE, 1932. Quantitative Clinical Chemistry. Volume I. Interpretations. Volume II. Methods. Baltimore.
- REDFIELD, ALFRED C., 1933. The evolution of the respiratory function of the blood. *Quart. Rev. Biol.*, **8**: 31-57.
- ROOT, R. W., 1931. The respiratory function of the blood of marine fishes. *Biol. Bull.*, **61**: 427-456.
- ROOT, R. W., AND LAURENCE IRVING, with the assistance of Virginia Safford and Henry Brown, 1940. The influence of oxygenation upon the transport of CO₂ by the blood of marine fish. *Jour. Cell. and Comp. Physiol.*, **17**: 85-96.
- ROUGHTON, F. J. W., 1935. Recent work on carbon dioxide transport by the blood. *Physiol. Rev.*, **15**: 241-296.
- VAN SLYKE, DONALD D., 1921. The carbon dioxide carriers of the blood. *Physiol. Rev.*, **1**: 141-176.